09/023,483

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Search Results - Record(s) 1 through 10 of 12 returned.

☐ 1. 5834285 . 04 Apr 94; 10 Nov 98. Recombinant thermostable DNA polymerase from archaebacteria. Comb; Donald G., et al. 435/194; 435/252.3 435/252.33 435/69.1 536/23.2. C12N009/10 C12N015/54 C12N015/70.

☐ 2. 5756334 . 06 Jul 94; 26 May 98. Thermostable DNA polymerase from 9.degree.N-7 and methods for producing the same. Perler; Francine B., et al. 435/194; 435/252.3 435/252.33 435/320.1 435/69.1 536/23.2. C12N009/10 C12N015/11 C12N015/54 C12N015/74.

☐ 3. 5731171 . 23 Jul 93; 24 Mar 98. Sequence independent amplification of DNA. Bohlander; Stefan K.. 435/91.2; 435/6. C12P019/34 C12Q001/68.

☐ 4. 5595871 . 25 Aug 93; 21 Jan 97. Detection and prevention of mycoplasma hominis infection. DelVecchio; Vito G., et al. 435/6; 435/252.3 435/320.1 435/91.2 435/91.5 536/23.1 536/24.32 536/24.33. C12Q001/68 C12P019/34 C07H021/04.

☐ 5. 5500363 . 07 Sep 93; 19 Mar 96. Recombinant thermostable DNA polymerase from archaebacteria. Comb; Donald G., et al. 435/194; 435/252.3 435/320.1 435/69.1. C12N009/10 C12N015/11 C12N015/54.

☐ 6. 5352778 . 15 Dec 93; 04 Oct 94. Recombinant thermostable DNA polymerase from archaebacteria. Comb; Donald G., et al. 536/23.2; 435/194 435/252.3 435/252.33 435/320.1 435/6 435/69.1 536/23.7 536/24.32. C12N009/10 C12N015/11 C12N015/54 C12N015/00.

☐ 7. 5322785 . 17 Apr 91; 21 Jun 94. Purified thermostable DNA polymerase obtainable from thermococcus litoralis. Comb; Donald G., et al. 435/194; 435/193 435/252.3 435/252.33 435/320.1 435/69.1 536/23.2. C12N015/54 C12N015/67 C12N015/70 C12N015/74.

☐ 8. 5242820 . 06 Jun 91; 07 Sep 93. Pathogenic mycoplasma. Lo; Shyh-Ching. 435/252.1; 435/5 435/872. C12N005/00 C12N005/02 C12N001/00 C12Q001/70.

☐ 9. 5210036 . 26 Apr 90; 11 May 93. Purified thermostable DNA polymerase obtainable from thermococcus litoralis. Comb; Donald G., et al. 435/194; 435/193. C12N009/12 C12N009/10.

☐ 10. 5106727 . 13 Jul 90; 21 Apr 92. Amplification of nucleic acid sequences using oligonucleotides of random sequences as primers. Hartley; James L., et al. 435/6; 435/5 435/810 435/91.2 435/91.21 436/501 436/94. C12Q001/68 C12P019/34 G01N033/48 G01N033/566.

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Search Results - Record(s) 11 through 12 of 12 returned.

☐ 11. 5043272 . 27 Apr 89; 27 Aug 91. Amplification of nucleic acid sequences using oligonucleotides of random sequence as primers. Hartley; James L.. 435/5; 435/6 435/810 435/91.2 436/501 436/94. C12P019/34 C12Q001/68 G01N033/566 G01N033/48.

☐ 12. 4965190 . 31 Jul 86; 23 Oct 90. Methods for the identification of mutations in the human phenylalanine hydroxylase gene using DNA probes. Woo; Savio L. C., et al. 435/6; 435/810 536/23.2 536/24.3 536/24.31. C12Q001/68 C07H019/073 C07H019/173.

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Term	Documents
PCR.DWPI,EPAB,JPAB,USPT.	31142
PCRS.DWPI,EPAB,JPAB,USPT.	861
(2 AND PCR).USPT,JPAB,EPAB,DWPI.	12
(L2 AND PCR).USPT,JPAB,EPAB,DWPI.	12

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L3: Entry 1 of 12 File: USPT

DOCUMENT-IDENTIFIER: US 5834285 A
TITLE: Recombinant thermostable DNA polymerase from archaebacteria

Brief Summary Text (6):
Recently, U.S. Pat. Nos. 4,683,195, 4,683,202 and 4,8000,159 disclosed the use of the above enzymes in a process for amplifying, detecting, and/or cloning nucleic acid sequences. This process, commonly referred to as polymerase chain reaction (PCR), involves the use of a polymerase, primers and nucleotide triphosphates to amplify existing nucleic acid sequences.

Brief Summary Text (9):
U.S. Pat. No. 4,889,818 discloses a purified thermostable DNA polymerase from T. aquaticus, Taq polymerase, having a molecular weight of about 86,000 to 90,000 daltons prepared by a process substantially identical to the process of Kaledin with the addition of the substitution of a phosphocellulose chromatography step in lieu of chromatography on single-strand DNA-cellulose. In addition, European Patent Application 0258017 discloses Taq polymerase as the preferred enzyme for use in the PCR process discussed above.

Brief Summary Text (10):
Research has indicated that while the Taq DNA polymerase has a 5'-3' polymerase-dependent exonuclease function, the Taq DNA polymerase does not possess a 3'-5' proofreading exonuclease function. Lawyer, F. C., et al. J. Biol. Chem., (1989) 254:11, p. 6427-6437. Bernard, A., et al. Cell (1989) 59:219. As a result, Taq DNA polymerase is prone to base incorporation errors, making its use in certain applications undesirable. For example, attempting to clone an amplified gene is problematic since any one copy of the gene may contain an error due to a random misincorporation event. Depending an where in the replication cycle that error occurs (e.g., in an early replication cycle), the entire DNA amplified could contain the erroneously incorporated base, thus, giving rise to a mutated gene product. Furthermore, research has indicated that Taq DNA polymerase has a thermal stability of not more than several minutes at 100.degree. C.

Detailed Description Text (26):
T. litoralis DNA can be used to construct genomic libraries as either random fragments or restriction enzyme fragments. The latter approach is preferred. Preferably, Eco RI partials are prepared from T. litoralis genamic DNA using standard DNA restriction techniques such as described in Maniatis, et al., Molecular Cloning: A Laboratory Manual (1982), the disclosure of which is incorporated herein by reference. Other restriction enzymes such as BamHI, NruI and XbaI can also be used.

Detailed Description Text (56):
In general, DNA from other archaebacterium can be isolated using the method described above. As with T. litoralis The archaebacterium DNA once isolated can be used to construct genamic libraries as either random fragments or restriction enzyme fragments. The latter approach is preferred. This approach generally entails cutting the target genomic DNA with various restriction enzymes and probing the fragments so formed with, for example, a T. litoralis DNA probe. A library is thereafter formed from one or more of the enzymes which produce a single hybridization band and which are about 4 Kb or large enough to at least code for the molecular weight of the target DNA polymerase.

Detailed Description Text (208):
Performance of T. Litoralis DNA Polymerase in the PCR Process

Detailed Description Text (209):

The ability of the T. litoralis DNA polymerase to perform the polymerase chain reaction (PCR) was also examined. In 100 .mu.l volumes containing the buffer described in Example IV, varying amounts of M13mp18 DNA cut by ClaI digestion, generating 2 fragments of 4355 bp and 2895 bp, were incubated with 200 ng of calf thymus DNA present as carrier DNA to decrease any nonspecific adsorption effects. The forward and reverse primers were present at 1 .mu.M (forward primer=5'd (CCAGCAAGGCCGATAGTTTGAGTT)3') (SEQ ID NO: 6) and the reverse primer=5' d(CGCCAGGGCTTTTCCCAGTCACGAC)3') (SEQ ID NO: 7). These primers flank a 1 kb DNA sequence on the 4355 bp fragment described above, with the sequence representing 14% of the total M13mp18 DNA. Also present were 200 .mu.M each dNTP, 100 .mu.g/ml BSA, 10% OMSO and 2.5 units of either T. aquaticus DNA polymerase (in the presence or absence of 0.5% NP40 and 0.05% Tween 20), or T. litoralis DNA polymerase (in the presence or absence of 0.10% Triton X-100). The initial cycle consisted of 5 min at 95.degree. C., 5 min at 50.degree. C. (during which polymerase and BSA additions were made) and 5 min at 70.degree. C. The segments of each subsequent PCR cycle were the following: 1 min at 93.degree. C., 1 min at 50.degree. C. and 5 min at 70.degree. C. After 0, 13, 23 and 40 cycles, 20 .mu.l amounts of 100 .mu.l volumes were removed and subjected to agarose gel electrophoresis with ethidium bromide present to quantitate the amplification of the 1 kb DNA sequence.

Detailed Description Text (211):
However, it was at the lower levels of target DNA sequence, 2.8 femtograms, that differences in polymerase function were most apparent. Under these conditions requiring maximal polymerase stability and/or efficiency at elongation of DNA during each cycle, the T. litoralis DNA polymerase produced greater than fourfold more amplified DNA than that of T. aquaticus DNA polymerase within 23 cycles.

Detailed Description Text (212):
This ability to amplify very small amounts of DNA with fewer cycles is important for many applications of PCR since employing large cycle numbers for amplification is associated with the generation of undesirable artifacts during the PCR process.

Detailed Description Text (235):
To facilitate this construction, a ScaI site was created in the PCR primers by changing the codon usage for Lys 1076 and Val 1077 as follows:

Detailed Description Text (238):
2) An about 638 bp fragment with ScaI and EcoRI termini derived from a PCR product. The reaction mixture contained 1X NEB Vent Polymerase Buffer, 0.1 mg/ml bovine serum alumen, 0.2 mM dNTPs (equimolar, each nucleotide), 0.9 .mu.g/ml pV174.1B1 plasmid DNA template, and 0.01 A.sub.260 U/ml of primer 72-150 (5' ATAAAGTACTTTAAAGCCGAACTTTTCCTCTA3') (SEQ ID NO: 21)and primer "JACK" (5' CGGCGCATATGATACTGGACACTGATTAC3'). 0.1 ml of the reaction mix was placed into each of five tubes, and the samples heated to 95.degree. C. for 3-5 minutes in a Perkin-Elmer Thermocycler. 1 U of Vent DNA polymerase was added to each reaction tube, and 15 cycles were run on the thermocycler consisting of 94.degree. C.- 0.5 minutes, 50.degree. C.- 0.5 minutes, and 72.degree. C.- 2 minutes. The samples were pooled, phenol extracted and ethanol precipitated. The sample was resuspended in 50 .mu.l Tris-EDTA buffer and mixed with 40 .mu.l of dH.sub.2 O, 10 .mu.l Of 10X NEBuffer 3, 60 units of ScaI endonuclease and 60 units of EcoRI endonuclease. After incubation at 37.degree. C. for 1.75 h, the reaction products were separated on a 1.5% agarose gel and the ca. 638 bp fragment was electroeluted, and quantified as described above.

Detailed Description Text (239):
3) An about 358 bp fragment with HindIII and ScaI termini derived from a PCR product. The reaction mixture contained 1 X NEB Vent Polymerase Buffer, 0.1 mg/ml bovine serum albumin, 0.2 mM dNTPs (equimolar, each nucleotide), 0.9 .mu.g/ml pV174.1B1 plasmid DNA template, and 0.02 A.sub.260 U/ml of primer 698 (5'GAGACTCGCGGAGAAACTTGGACT3') (SEQ ID NO: 23) and primer 73-143 (5'TACAGTACTTTATGCGGACACTGACGGCTTTTATGCCAC3') (SEQ ID NO: 24). 0.1 ml of the reaction mix was placed into each of five tubes, and the samples heated to 95.degree. C. for 3-5 minutes in a Perkin-Elmer Thermocycler. 1 U of Vent DNA polymerase was added to each reaction tube, and 20 cycles were run on the thermocycler consisting of 94.degree. C.- 0.5 minutes, 50.degree. C.- 0.5 minutes, and 72.degree. C.-1 minute. The samples were pooled, phenol extracted and ethanol precipitated. The sample was resuspended in 50 .mu.l Tris-EDTA buffer and cleaved with

HinduIII and ScaI endonucleases. The reaction products were separated on a 1.5% agarose gel and the 358 bp fragment was electroeluted, and quantified as described above.

Detailed Description Text (250):
In order to determine which restriction enzymes would be most useful in preparation of the Pyrococcus genomic library, Pyrococcus sp. DNA was cut to completion with Eco RI, BamHI and HindIII. This DNA was subject to agarose gel electrophoresis (FIG. 13A) and Southern hybridization (FIG. 13B) using a DNA probe prepared as follows. A reaction mixture containing 1 .mu.g of the first EcoRI fragment of the T. litoralis DNA polymerase gene (bp 1-1274, obtainable ,from bacteriophage NEB#618, ATCC No. 40794) as a template in a commercial random priming kit (New England Biolabs, Inc.) was incubated for 1 hour at 37.degree. C. to produce a DNA probe of high specific activity. The probe was hybridized to Pyrococcus sp. DNA prepared above under moderately stringent conditions (Hybridization: overnight at 50.degree. C., 4X SET, 0.1 M sodium phosphate, pH 7, 0.1% Na pyrophosphate, 0.1% SDS, 1X Denhardts solution; Wash Conditions: wash 3X 20-30 min. 45.degree. C., 0.1X SET, 0.1 M sodium phosphate, (pH 7), 0.1% Na pyrophosphate, 0.1% SDS. Maniatis, et al., supra). A single major band at about 5 Kb was detected in BamH I cut Pyrococcus DNA. EcoR I and Hind III gave multiple bands with this probe, indicating that these enzymes cut within the Pyrococcus polymerase gene.

Detailed Description Text (251):
Based on these results, a BamHI genomic library was constructed using the phage vector .lambda.DASH (Stratagene). Partial and complete BamHI digests of Pyrococcus DNA were prepared. A mixture of the partial and completely BamHI digested DNA was ligated into the BamHI site or .lambda.DASH. The ligation mixture was packaged using Gigapack Gold (Stratagene) according to manufacturer's instructions and plated an E. coli ER1458. The packaged phage library contained 1.times.10.sup.6 phage per ml. .sup.32 P-labelled DNA probes of the 3 fragments (bp 1-1274, 1656-2660 and 3069-3737) of the T. litoralis DNA polymerase gene (obtainable from NEB#619, ATCC No. 40795) were prepared using a random primer kit (New England Biolabs, Inc.). The probes were used according to the method of Benton & Davis (Maniatis, et al. supra) to screen the Pyrococcus genomic library using hybridization conditions described above. About one per cent of the plaques were positive and ten positive plaques were picked and purified by reinfection and replating 3 times (until 90-100% of the plaques were positive or each isolate). Large amounts of phage were prepared from each isolate and used to infect E. coli cultures. Specifically, plate lysates (Maniatis et al. supra) of phage were prepared from each isolate and used to infect E. coli cells. 0.1 ml of each plate lysate was mixed with E. coli with 0.2 ml of cells (OD.sub.600 =2). The bacterial cells were harvested just before lysis and suspended in 0.05 M NaCl, 0.01 M Tris (pH 8.0), 0.1 mM EDTA, 0.1% Triton X-100 and 200 .mu.g/ml lysozyme (3 volumes per volume of cells) and heated to 37.degree. C. for about 1 minute or until cell lysis occurred. The lysed extracts were immediately heated at 75.degree. C. for 30 minutes, centrifuged and the supernatant solution assayed for heat stable DNA polymerase activity, according to the method described above. Three of the ten isolates showed significant polymerase activity and the clone (B9) showing the most activity was investigated further.

Detailed Description Text (259):
Four gel purified fragments of T. litoralis DNA polymerase DNA, (1.3 kb Eco RI fragment from bp 1-1274 representing the 5' polymerase coding region; bp 4718-5437, representing the 3' polymerase coding region; bp 2448-2882, representing part of IVS1; and bp 3666-4242, representing part of IVS2, FIGS. 6 and 15) were radiolabelled using the New England Biolabs Random Primer Kit. 10 ng of the above template DNAs, each in a volume of 35.5 .mu.l, were boiled for 5 minutes in a boiling water bath and then cooled on for 5 minutes and spun down. The template DNAs were incubated wit 1X labelling buffer (includes random hexanucleotides), 1/10 volume dNTP mix, 25 .mu.Ci .sigma..sup.32 p dCTP and 5 units DNA Polymerase r-Klenow fragment in a total volume of 50 .mu.l for 1 hour at 37.degree. C. The reactions were stopped with 0.18 M EDTA. The probes were purified using an Elutip minicolumn (Schleicher and Schuell) following the manufacturers recommended elution conditions. The total number of counts were calculated for all purified probes. The 1.3 kb Eco RI fragment probe (bp 1-1274) yielded 24.times.10.sup.6 cpm, the 3' polymerase probe (bp 4718-5436) yielded 22.times.10.sup.6 cpm, the IVS1 probe yielded 54.times.10.sup.6 cpm, and the IVS2 probe yielded 47-10.sup.6 cpm.

Other Reference Publication (54):
Flaman, J.-M., et al., Nucleic Acids Research, vol. 22, "A rapid PCR fidelity assay", pp. 3259-3260, 1994.

Other Reference Publication (56):
Cline, J., et al., Nucleic Acids Research, vol. 24, "PCR fidelity of Pfu DNA polymerase and other thermostable DNA polymerases", pp. 3546-3551, 1996.